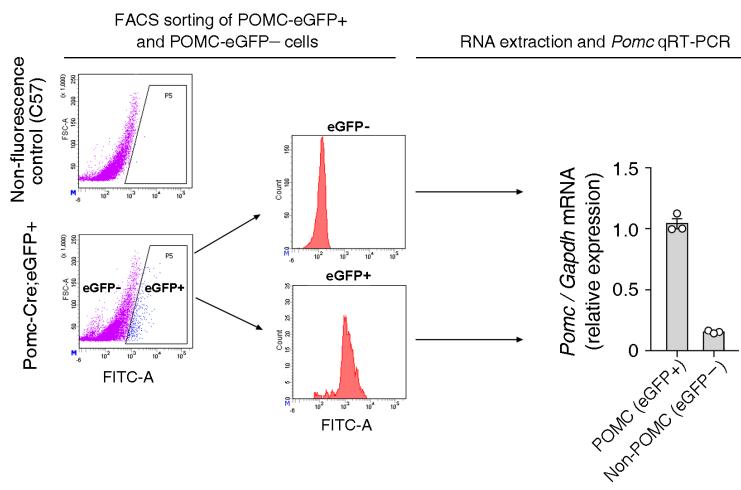
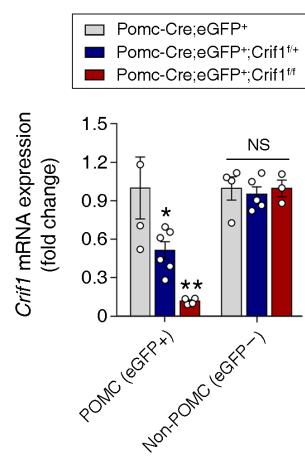


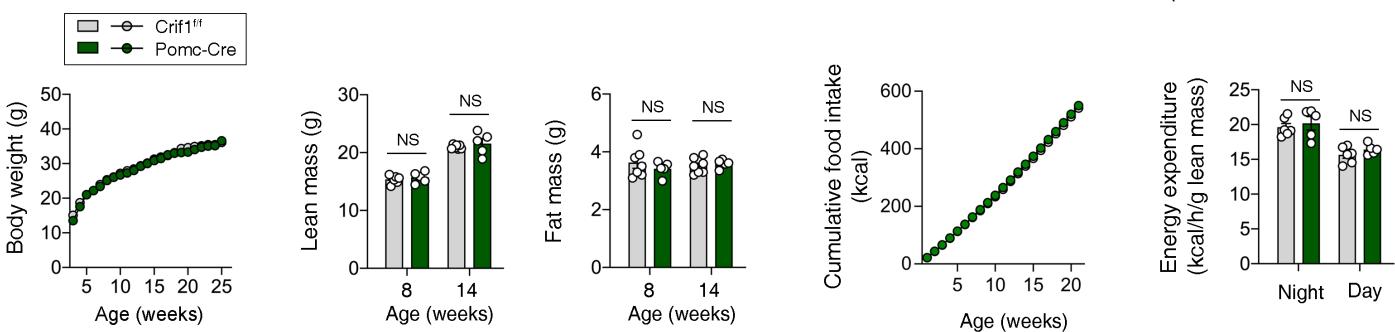
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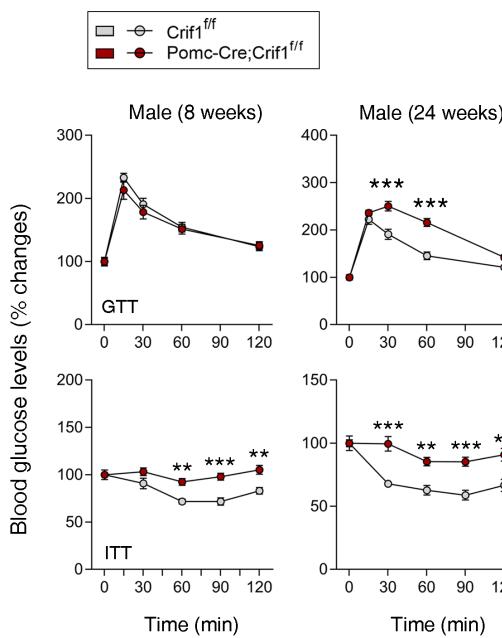
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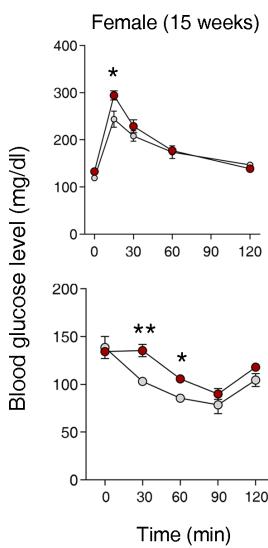
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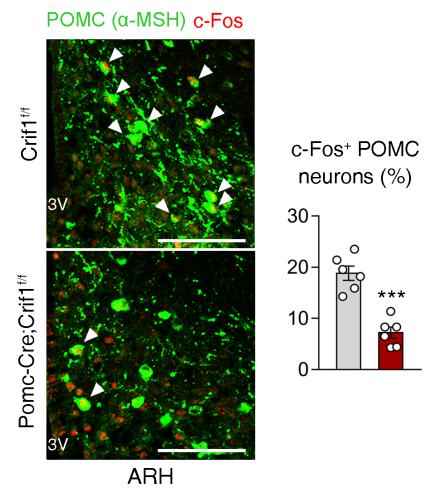
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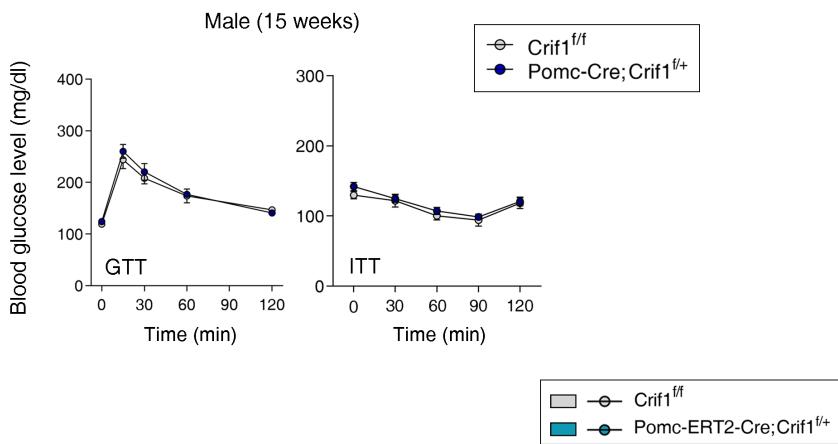
**Figure S1. Confirmation of POMC neuronal *Crif1* depletion and metabolic phenotyping of *Crif1<sup>ff</sup>*, *Pomc-Cre*, and *Pomc-Cre; Crif1<sup>ff</sup>* mice.**

**Related to Figure 1**

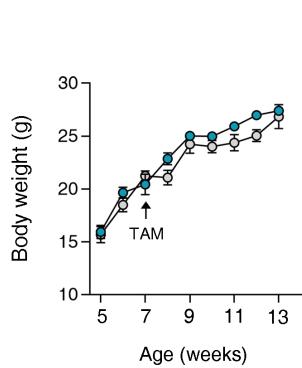
- (A) The representative dot plot depicting FACS sorting of eGFP+ and eGFP- cells isolated from the hypothalamus of *Pomc-Cre; eGFP+* mice and confirmation of successful FACS sorting using real-time PCR analysis of *Pomc* expression in eGFP+ and eGFP- cells.
- (B) Comparison of *Crif1* mRNA expression among the mice groups in hypothalamic POMC-eGFP+ and POMC-eGFP- cells ( $n = 3-6$ ).
- (C) No differences in body weight, lean and fat mass, cumulative food intake, and energy expenditure between *Pomc-Cre* mice and *Crif1<sup>ff</sup>* mice ( $n = 5-7$ ).
- (D) The percentage changes in blood glucose levels during glucose and insulin tolerance tests (GTT, ITT) in *Pomc-Cre; Crif1<sup>ff</sup>* male mice and their *Crif1<sup>ff</sup>* littermates at indicated ages ( $n = 5-8$ ).
- (E) GTT and ITT performed in *Crif1<sup>ff</sup>* and *Pomc-Cre; Crif1<sup>ff</sup>* female mice at 15 weeks of age ( $n = 5-7$ ).
- (F) POMC (α-MSH) and c-Fos double staining in 14-week-old *Pomc-Cre; Crif1<sup>ff</sup>* mice showing reduced POMC neuronal activity ( $n = 6$ ). 3V, 3rd cerebroventricle; ARH, hypothalamic arcuate nucleus. Scale bars, 100 μm.

Results presented as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$  vs. *Pomc-Cre; eGFP+* or *Crif1<sup>ff</sup>* littermates. NS: not significant.

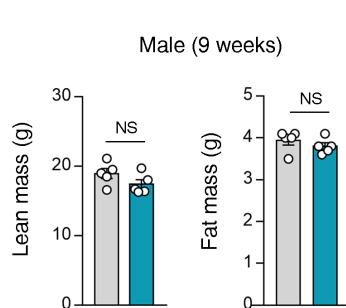
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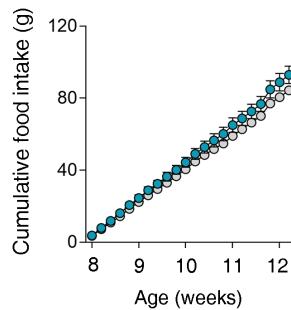
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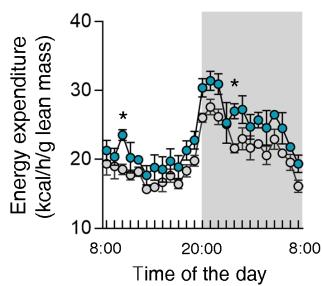
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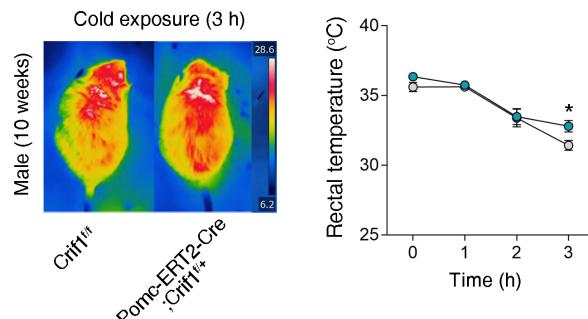
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**Figure S2. Metabolic phenotype analysis of mice with *Crif1* partial deficiency in POMC neurons. Related to Figure 2**

(A) Glucose tolerance test (GTT) and insulin tolerance test (ITT) in 15 week-old Crif1<sup>t/t</sup> and Pomc-Cre; Crif1<sup>t/+</sup> male mice ( $n = 4\text{--}6$ ).

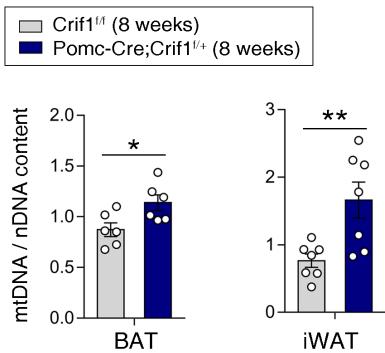
(B-E) Body weights, lean and fat mass, food intakes, and energy expenditure in mice with adult-onset POMC neuron-specific Crif1 partial deficiency.

Gene deletion was induced by intraperitoneal injection of tamoxifen (TAM, 75 mg/kg for 5 days) at 7 weeks of age ( $n = 6$ ).

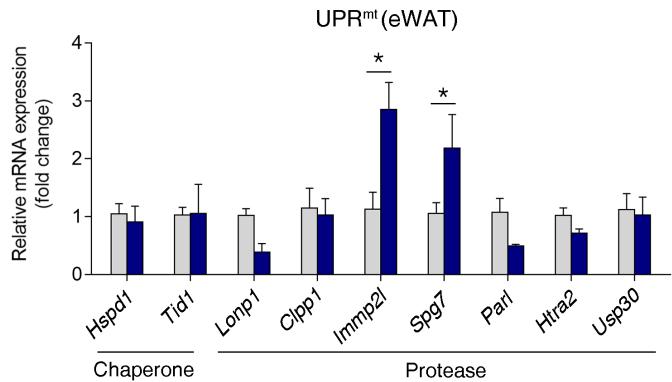
(F) Skin and rectal temperature during 4 °C cold exposure ( $n = 6$ ).

Results presented as mean  $\pm$  SEM. \* $p < 0.05$  vs. Crif1<sup>t/t</sup> littermates. NS: not significant.

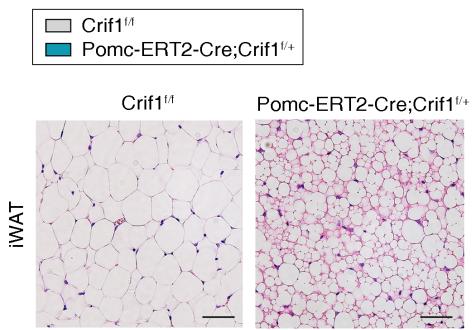
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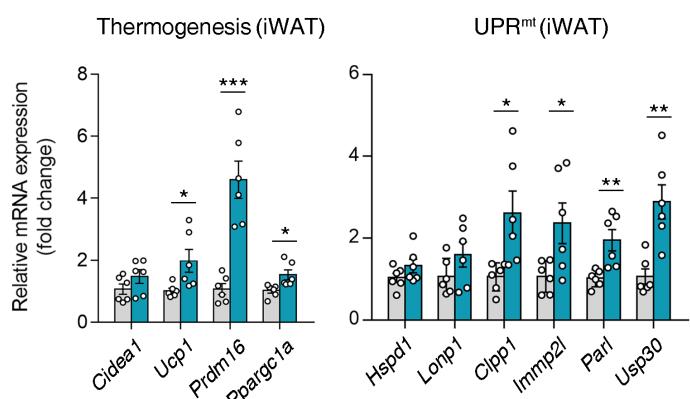
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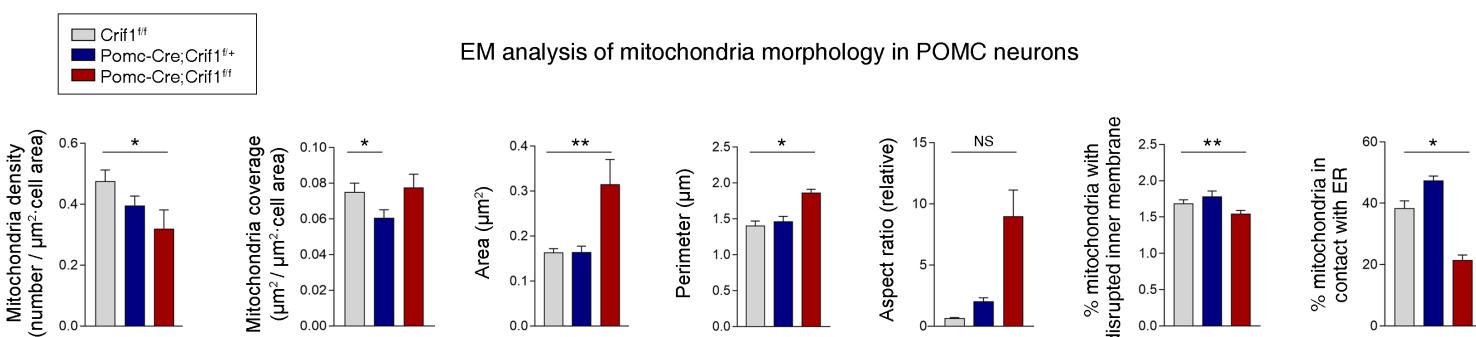
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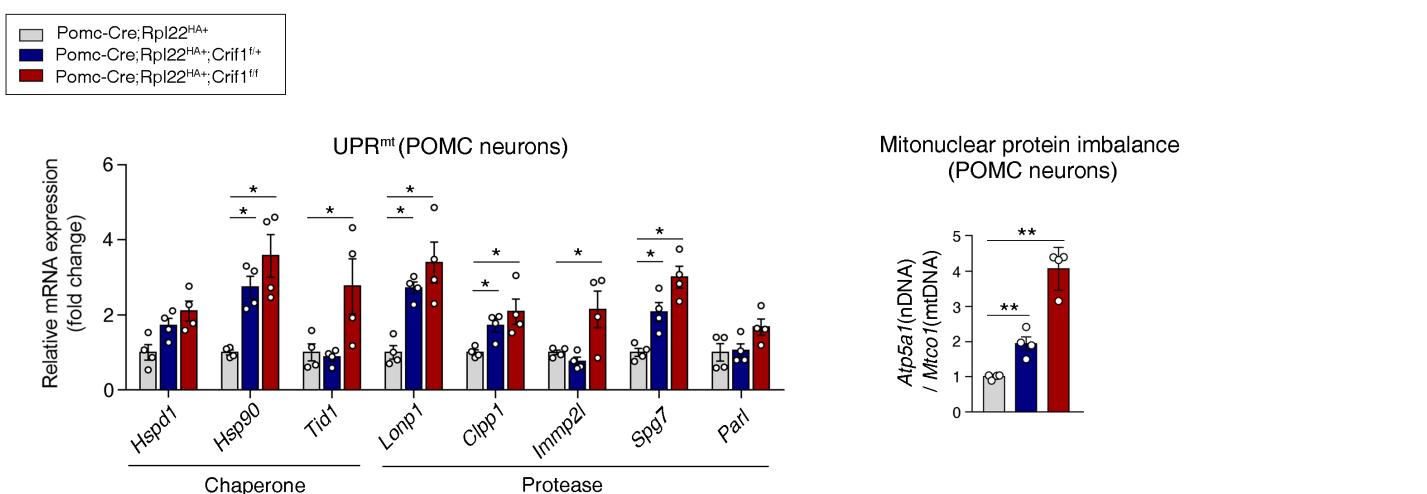
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**Figure S3. Adipose tissue and POMC neuronal mitochondrial stress responses in mice with POMC-specific *Crif1* deficiency.** Related to Figure 3 (A) The mtDNA / nDNA ratio in BAT and iWAT of 8-week-old Crif1<sup>fl/fl</sup> and Pomc-Cre; Crif1<sup>fl/+</sup> male mice ( $n = 6-7$ ).

(B) Mild mitochondrial unfolded protein responses (UPR<sup>mild</sup>) in the epididymal adipose tissue (eWAT) of Pomc-Cre; Crif1<sup>fl/+</sup> male mice ( $n = 6$ ).

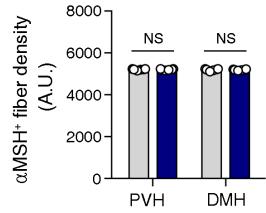
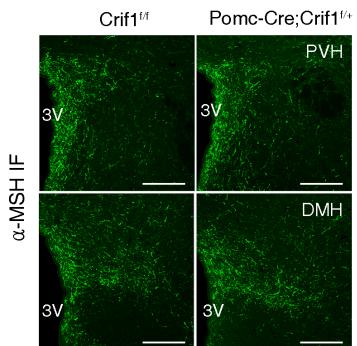
(C, D) BAT-like changes, thermogenesis-related gene expression, and UPR<sup>mild</sup> in iWAT of 11-week-old mice with adult (at 7 weeks)-induced *Crif1* partial deletion in POMC neurons ( $n = 6$ ). Scale bars, 50  $\mu$ m.

(E) Mitochondrial analysis in *Crif1* deficient POMC neurons using POMC immune-EM in 11 weeks-old mice ( $n = 5$ ).

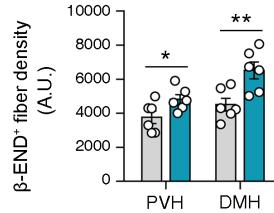
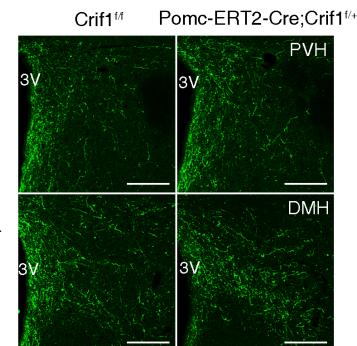
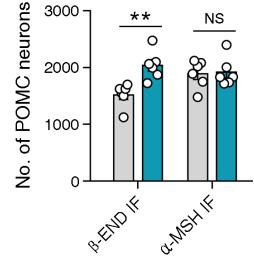
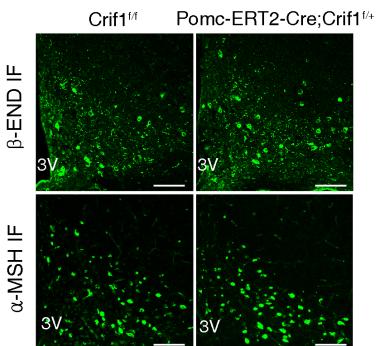
(F) Measurement of UPR<sup>mild</sup> and mitonuclear protein imbalance in *Crif1*-depleted POMC neurons using RiboTag (Rpl22<sup>HA</sup>) mice at 7–8 weeks ( $n = 4$ ).

Results presented as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$  between indicated groups. NS: not significant.

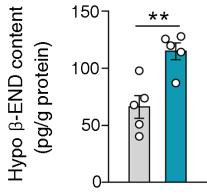
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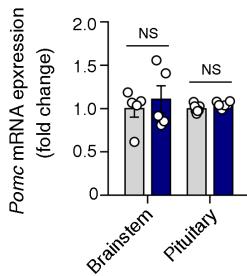
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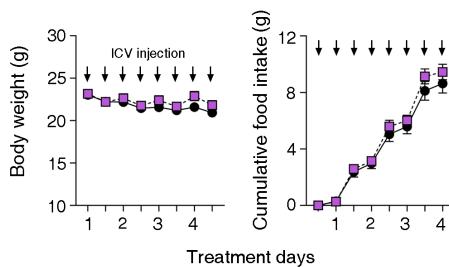
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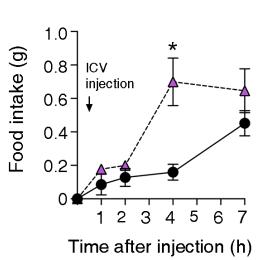
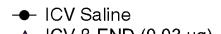
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**Figure S4. Increase in hypothalamic β-END expression by adult-induced Crif1 heterodeficiency in POMC neurons. Related to Figure 4**

(A) The α-MSH<sup>+</sup> axonal fiber density in the hypothalamic PVH and DMH of POMC-Cre; Crif1<sup>t/t</sup> mice and Crif1<sup>t/t</sup> littermates ( $n = 6$ ). Scale bars, 100 µm.

(B) Increased β-END<sup>+</sup> neuron numbers and axonal fiber density, but unaltered α-MSH<sup>+</sup> neuron numbers in POMC-ERT2-Cre; Crif1<sup>t/t</sup> mice ( $n = 6$ ).

Gene knockout was induced by injecting tamoxifen at 7 weeks. Scale bars, 100 µm.

(C) Increased hypothalamic β-END contents in POMC-ERT2-Cre; Crif1<sup>t/t</sup> mice ( $n = 5$ ).

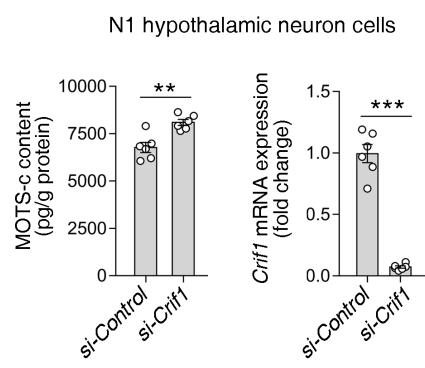
(D) POMC mRNA expression in the brainstem and pituitary gland of POMC-Cre; Crif1<sup>t/t</sup> mice and Crif1<sup>t/t</sup> littermates ( $n = 5$ ).

(E) Measurements of body weight and cumulative food intake during repeated ICV injection of saline or β-END (0.1 µg twice a day for 4 days) ( $n = 8–10$ ). Arrows indicate times of ICV injection.

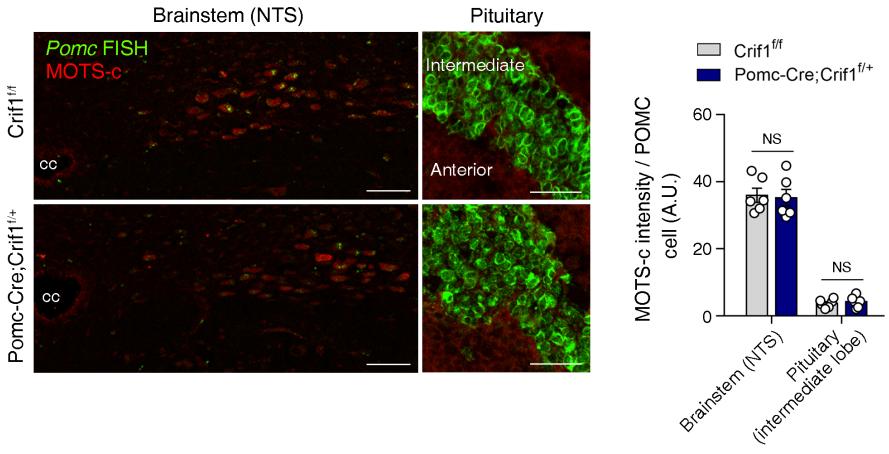
(F) Food intakes following single ICV injection of β-END in freely-fed C57 mice ( $n = 4–5$ ).

Results presented as mean ± SEM. \* $p < 0.05$ , \*\* $p < 0.01$  between indicated groups or ICV saline. NS: not significant.

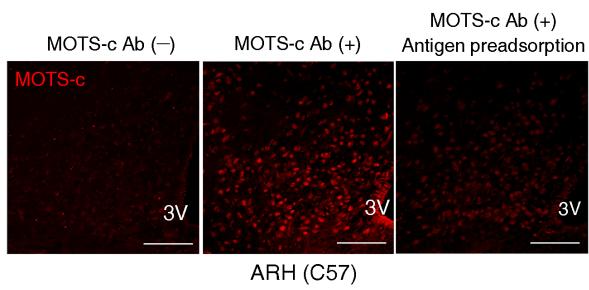
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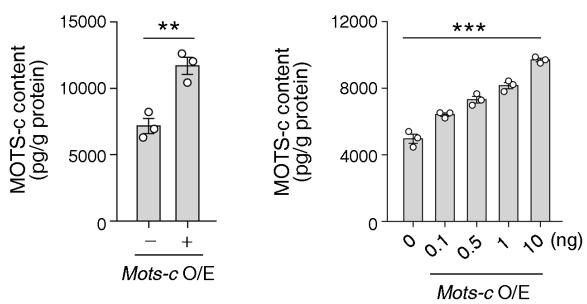
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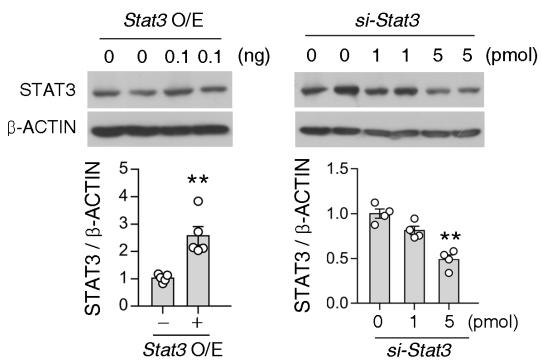
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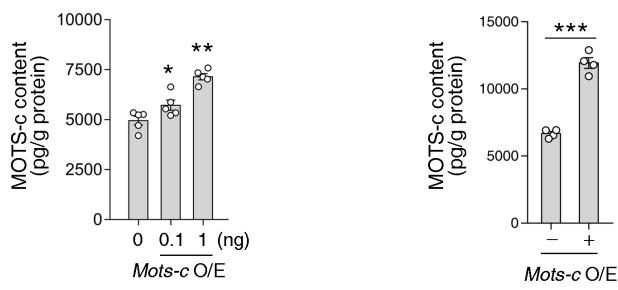
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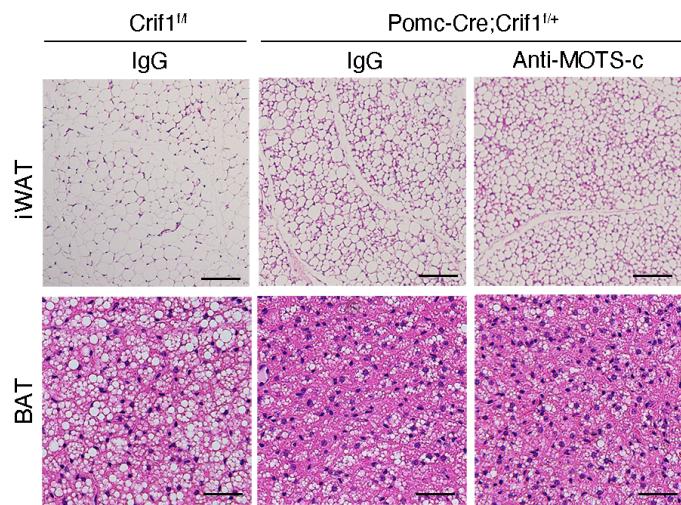
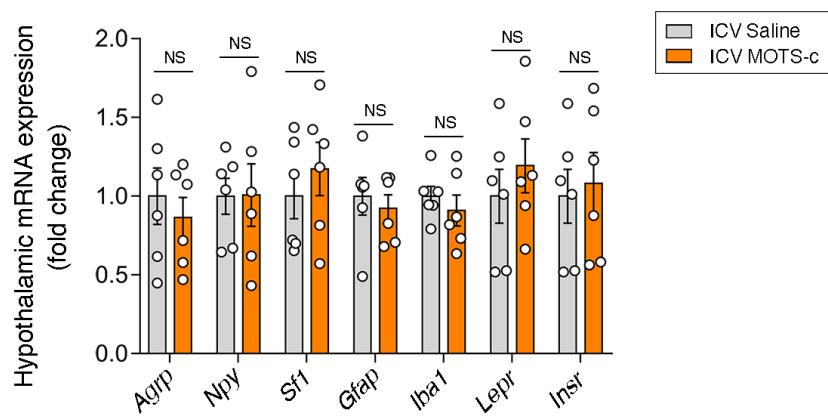
F



**Figure S5. Involvement of MOTS-c in mitoribosomal stress response of POMC neurons. Related to Figure 5**

- (A) *Crif1* knockdown with *Crif1* siRNA in N1 hypothalamic neuron cells increases cellular MOTS-c protein contents ( $n = 5$  wells).
- (B) *Pomc* FISH and MOTS-c immunofluorescence double staining showing MOTS-c expression in brainstem solitary tract nucleus (NTS) and pituitary POMC cells of *Crif1<sup>fl/fl</sup>* and *Pomc-Cre; Crif1<sup>fl/+</sup>* mice ( $n = 6$ ). cc, central canal. Scale bars, 50  $\mu$ m.
- (C) The MOTS-c antibody specificity test for hypothalamic MOTS-c immunohistochemistry using antigen preadsorption. Scale bars, 50  $\mu$ m.
- (D) Confirmation of *Mots-c* gene overexpression (O/E) by measuring cellular MOTS-c protein contents using ELISA ( $n = 3$ ).
- (E) Confirmation of *Stat3* overexpression/knockdown and *Mots-c* overexpression in *Pomc* promotor experiments ( $n = 4–6$ ).
- (F) Confirmation of *Mots-c* overexpression in Chip experiment ( $n = 4$ ).

Results presented as mean  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$  vs. controls. NS: not significant.

**A****B**

**Figure S6. Studies of ICV administration of MOTS-c peptide and neutralizing antibody. Related to Figure 6**

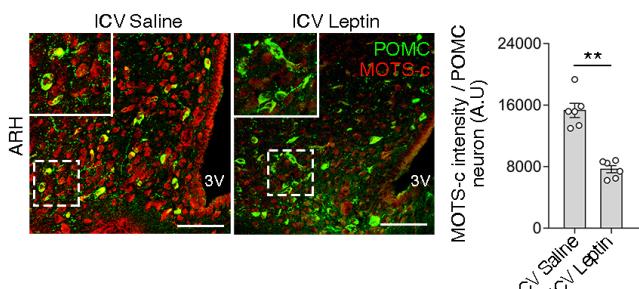
(A) Histology of iWAT and BAT in Pomc-Cre; Crif1<sup>fl/+</sup> mice receiving intra-ARH infusion of either IgG or anti-MOTS-c antibody for 7 days.

As a control, Crif1<sup>fl/fl</sup> mice were infused with IgG in their ARH ( $n = 4\text{--}5$ ). Scale bars, 50  $\mu\text{m}$ .

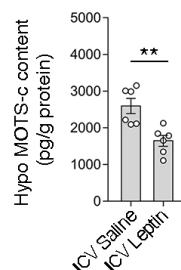
(B) No alteration in the hypothalamic expression levels of neuronal markers, glial markers, and hormonal receptors by ICV MOTS-c treatment ( $n = 6$ ).

Results presented as mean  $\pm$  SEM. NS: not significant.

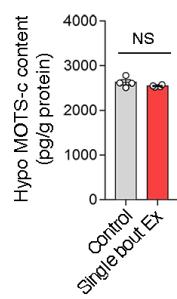
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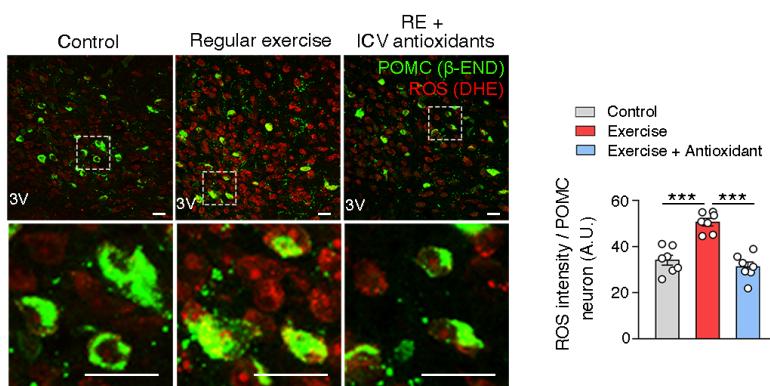
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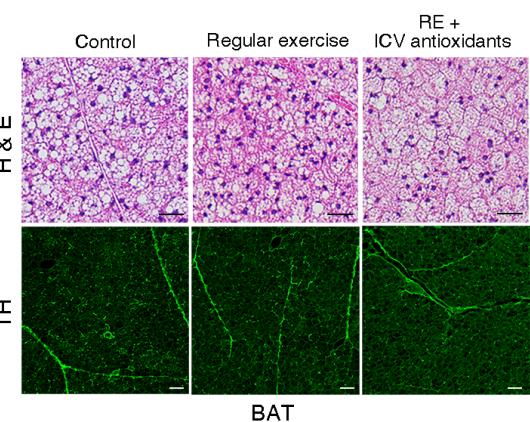
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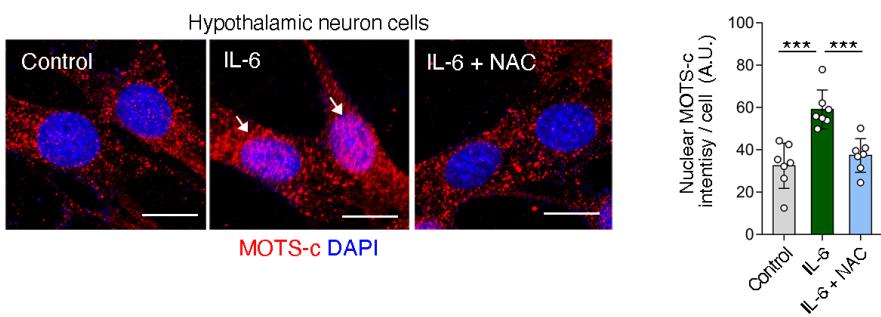
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**Figure S7. Effects of leptin, IL-6, and single-bout exercise on hypothalamic MOTS-c expression. Related to Figure 7**

(A, B) MOTS-c / POMC ( $\alpha$ -MSH) immunohistochemistry and MOTS-c ELISA assay showing a reduction in hypothalamic MOTS-c expression following ICV leptin administration ( $n = 6$ ). Scale bars, 50  $\mu$ m.

(C) No alteration in hypothalamic MOTS-c content after single bout high-intensity running exercise ( $n = 5$ ).

(D) ROS (DHE) and  $\beta$ -END double staining in the hypothalamus of mice which underwent moderate-intensity regular running exercise with or without ICV antioxidant treatment ( $n = 7$ ). Scale bars, 20  $\mu$ m.

(E) No significant changes in the BAT histology and sympathetic innervation after 2 week-regular running exercise ( $n = 7$ ). Scale bars, 50  $\mu$ m (upper) and 200  $\mu$ m (lower).

(F) MOTS-c nuclear translocation upon treatment of IL-6 +/- antioxidant N-acetyl cysteine (NAC) in N1 hypothalamic neuron cells ( $n = 10$  wells). Scale bars, 25  $\mu$ m. Arrows indicate nuclear MOTS-c expression.

Results presented as mean  $\pm$  SEM. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  between indicated groups. NS: not significant.

**Table S1. Primer sequences used for real time PCR analysis. Related to STAR METHODS**

Gene	Forward	Reverse
<i>Agrp</i>	5'-ACAACTGCAGACCGAGCA-3'	5'-GACGCGGAGAACGAGACT-3'
<i>Atp23</i>	5'-GACTGCTCCCTTGTGAACGA-3'	5'-CGCACGCAAGTCTGATGATG-3'
<i>Atp5a1</i>	5'-CATTGGTGATGGTATTGCCG-3'	5'-TCCCAAACACGACAACCTCC-3'
<i>Cidea1</i>	5'-TGCTCTCTGTATGCCAGT-3'	5'-GCCGTGTTAAGGAATCTGCTG-3'
<i>Clpp1</i>	5'-GCCATTCACTGCCAATTCC-3'	5'-TGCTGACTCGATCACCTGTAG-3'
<i>Cpe</i>	5'-CAGCAAGAGGACGGCATCTC-3'	5'-GTCCAACCGCCTCATTACCAT-3'
<i>Crif1</i>	5'-TATCTCCTGCGGCTCTGT-3'	5'-CTTCTGCTTCGCCAGTTT-3'
<i>Gapdh</i>	5'-CCTGTTGCTGTAGCCGTAT-3'	5'-ACTCTTCCACCTTCGATGC-3'
<i>Gfap</i>	5'-CACCTACAGGAAATTGCTGGAGG-3'	5'-CCACGATGTTCCCTTGTAGGTG-3'
<i>Hspd1</i>	5'-GAGCTGGGTCCCTCACTCG-3'	5'-AGTCGAAGCATTCTGCGGG-3'
<i>Hsp60</i>	5'-GACGTTGACGGAGAACGCTCAA-3'	5'-CACTGCACCACCAGTAGCAATA-3'
<i>Hsp90</i>	5'-AGGTCCCTCGGAGTCAACCAC-3'	5'-TCAAATTGTATGTCCGCCGT-3'
<i>Htra2</i>	5'-TCCCCGGAGCCAGTACAAT-3'	5'-GAAAGGGTGCCGGTCTAGG-3'
<i>Iba1</i>	5'-TCTGCCGTCCAAACTTGAAGCC-3'	5'-CTCTTCAGCTCTAGGTGGGTCT-3'
<i>Immp1l</i>	5'-ATGACCCATGCACGCTTGA-3'	5'-TCTGCTACCACCAGCCATAA-3'
<i>Immp2l</i>	5'-ACATGTGGGTTGAAGGCGAT-3'	5'-CCCAGAGAAACCGGTCCAAA-3'
<i>Insr</i>	5'-AGATGAGAGGTGCACTGTGGCT-3'	5'-GGTCCTTGGCTTGCACACA-3'
<i>Lepr</i>	5'-CTTCCTGTGGACAGAACCGAC-3'	5'-AGCACTGAGTGAETCCACAGCA-3'
<i>Lonp1</i>	5'-AGCCCTATGTTGGCGTCTC-3'	5'-CCGGCTGATGTGAATCCTTCT-3'
<i>Mtco1</i>	5'-CCCAGATATAGCATTCCCACG-3'	5'-ACTGTTCATCCTGTTCCCTGC-3'
<i>mtDNA</i>	5'-AAGACACCTTGCCTAGCCACAC-3'	5'-TGGCTGGCACGAAATTAC-3'
<i>nDNA</i>	5'-AACTTCGATGGTAGTCGCCG-3'	5'-CCTGGATGTGGTAGCCGTT-3'
<i>Npy</i>	5'-GGACTGACCCCTCGCTCTA-3'	5'-TCGCAGAGCGGAGTAGTA-3'
<i>Pam</i>	5'-CTGGGGTCACACCTAAAGAGT-3'	5'-ATGAGGGCATGTTGCATCCAA-3'
<i>Parl</i>	5'-TCACCATTGGGTTCACAGGC-3'	5'-TCCTTTGTGGCCGTATGCT-3'
<i>Pcsk1</i>	5'-AGTTGGAGGCATAAGAATGCTG-3'	5'-GCCTTCTGGCTAGTCTGC-3'
<i>Pcsk2</i>	5'-AGAGAGACCCCAGGATAAAGATG-3'	5'-CTTGCCAGTGTGAACAGGT-3'
<i>Pomc</i>	5'-CAGGT CCTGGAGTCCGAC-3'	5'-CATGAAGCCACCGTAACG-3'
<i>Ppargc1a</i>	5'-CCCTGCCATTGTTAACGACC-3'	5'-TGCTGCTGCTCCTGTTTC-3'
<i>Prep</i>	5'-CCGCATTGTCAGCCAGTC-3'	5'-CCAAAGTGGTCAACCTCTGTT-3'
<i>Prdm16</i>	5'-AGCTACTGGTGGAACGGAGA-3'	5'-TTAAGCCAATGCTCGCTTCT-3'
<i>Sfl</i>	5'-GTGCATGGTCTTAAGGAGCTGG-3'	5'-GGATGCTGTCTCCTTGCCTGA-3'
<i>Spg7</i>	5'-TGTCTGGGTTCTCCAACACG-3'	5'-GTGCCCATTCGGTCCATCTC-3'
<i>Tid1</i>	5'-GGAAGCAAGGATAGGCGAGA-3'	5'-GTTGACCGCTTCCTCAGCAG-3'
<i>Ucp1</i>	5'-AGGGCCCCCTTCATGAGGTC-3'	5'-GTGAAGGTCAGAATGCAAGC-3'
<i>Usp30</i>	5'-GAACTGGGAGTGTAGCGGTG-3'	5'-ACAAGCCCTTCTCCGCTT-3'
<i>18s</i>	5'-TCATAAGCTTGCCTGATTA-3'	5'-TAGTCAAGTTCGACCGTCTT-3'